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Neurochemistry and Behavior in Man

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The distribution and functions of certain neurotransmitter substances seem to correlate with clinical, anatomical and physiological evidence about the mediation of normal and abnormal behaviors in man, though much remains to be learned. The biosynthetic and metabolic pathways, receptors and reuptake mechanisms, and relationships to cyclic nucleotides for several major neurotransmitters are characterized, as well as the specific actions of many behavior-modifying drugs employed clinically. Experimental systems, including nerve cells in culture, permit tests of molecular actions inferred from biochemical and neurophysiological analyses in intact brain. This selective review emphasizes advances in neurochemistry which provide a context for current and future research on neurological and psychiatric disorders encountered in clinical practice.

THE CELLULAR AND BIOCHEMICAL organization and functions of the brain are under intensive investigation in man, as well as other species. The neurosciences are expanding rapidly, reflected in multidisciplinary research groups and in a burgeoning literature of journal articles and books.[1-9] Much has been learned about the roles of specific neurotransmitter molecules in anatomically and pathologically defined pathways in the brain. Meanwhile, the widespread clinical use of behavior-modifying drugs having defined biochemical actions has provided observations and generated hypotheses that relate neurochemical findings to normal and abnormal behaviors in man.

This review will emphasize principles of the organization and function of the nervous system, including the roles of specific neurotransmitters and their receptors, the involvement of cyclic nucleotides, and the phenomenon of disinhibition. Recent advances in biochemistry of depression, schizophrenia and opiate addiction will be highlighted.

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Principles of Organization

Nerve Cells Are Extremely Heterogeneous

Nervous tissue has two special properties: first, its electrically excitable cells, neurons, can conduct bioelectric signals over long distances without any loss of signal strength; second, these cells form specific intercellular connections with other nerve cells, muscle and glands, directing a grand array of bodily functions. Neurons are highly heterogeneous in size and shape. Neurons may have just one process, as in sensory ganglia and dorsal root ganglia, or two processes, as in bipolar sen-

ABBREVIATIONS USED IN TEXT

ACh = gamma-aminobutyric acid
AChR = nicotinic receptor for ACh
AChE = acetylcholinesterase
AMP = adenosine monophosphate
ATP = adenosine triphosphate
ChA = choline acetylase
COMT = catechol-O-methyltransferase
DA = dopamine
DBH = dopamine-beta-hydroxylase
DNA = deoxyribonucleic acid
dopa = dihydroxyphenylalanine
5HT = 5-hydroxytryptamine (serotonin)
GABA = gamma-aminobutyric acid
GAD = glutamic acid decarboxylase
GMP = guanosine monophosphate
LSD = lysergic acid diethylamide
MAO = monoamine oxidase
NE = norepinephrine
RNA = ribonucleic acid
TH = tyrosine hydroxylase

sory receptor cells of the retina, olfactory mucosa, auditory nerve and small granule cells within the brain. Most neurons are multipolar, with an axon of variable length and branching and many dendrites constituting a receptive field for the nerve cell body. Axons and dendrites form specialized nontouching connections called synapses, where much of the neurochemical story takes place.

With regard to neurochemistry, the interior of the neuron may be described briefly as having a nucleus and nucleolus, where the deoxyribonucleic acid (DNA) genetic material is carried and transcribed into ribonucleic acid (RNA); polyribosomes in the cell body, for active protein synthesis; mitochondria throughout the cell to support energy metabolism; microfilaments and microtubules extending down the axon through which metabolites and proteins are rapidly or slowly transported to the axon terminal at the synapse, and a metabolically extremely active terminus in which neurotransmitter molecules are stored in synaptic vesicles from which these molecules are released into the synapse upon electrical stimulation.

The brain contains some 100 billion cells, 90 percent of which are two types of neuroglia: (1) fibrous astrocytes, found in close approximation to axons and dendrites and blood vessels, are thought to insulate conducting surfaces of neurons and affect spatial distribution of neuronal processes; (2) oligodendrocytes form concentric layers of their own surface membrane around an axon, generating the axonal myelin sheath. Even those axons that lack a myelin sheath have an enclosing single invagination of oligodendrocyte surface membrane. Although glia have been presumed to "nurture" the neurons and their processes and possibly even be involved in modulation of the bioelectric signal or the formation of memory traces, no such functions have been demonstrated and axons which are cut off from a cell body cannot survive on glial support.

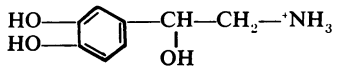
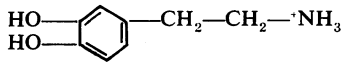
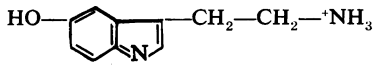
Finally, the vasculature of the brain and the blood-brain permeability barrier must be mentioned. The brain is extremely dependent upon oxidative metabolism and highly sensitive to lack of oxygen or glucose,[10] which are continuously distributed within the brain by the vascular system. The cerebral vasculature has a neurogenic mechanism to maintain blood flow even in the face of loss of volume or perfusion pressure elsewhere in the body.[11] The endothelial cells of brain capillaries differ from those in muscle and other tissues in having much more highly developed intercellular zones of membrane apposition without pinocytotic vesicles. Permeability barriers, when intact, protect the brain against blood-borne proteins and biological agents and also differentially admit lipophilic and keep out hydrophilic, charged small molecules, including many drugs and amino acid precursors. These barriers operate in both directions; thus, acid metabolites of monoamine neurotransmitters accumulate in the cerebrospinal fluid if probenecid is administered to block the acid transport system of the choroid plexus.

Neurons Make Connections Through Synapses: Neurotransmitters and Receptors

The synapse is a specialized contact zone between neuronal processes. The terminal portion of the axon is rich in microvesicles containing chemicals to be released as mediators of neurotransmission. Electron micrographs of the contact zone show dense material, presumably proteinaceous in nature, lining the intracellular portions of the presynaptic and postsynaptic membranes and filling the synaptic cleft between the apposed surfaces of the two cells. The material (neurostenin) consists of actomyosin-like proteins with Mg^{++}, Ca^{++}-activated adenosinetriphosphatase activity which may be involved in exocytosis, undergoing conformational changes with subsequent release of transmitter.[12]

The axon terminal is served by axoplasmic

TABLE 1.—*The Major Neurotransmitters*

Transmitter Molecule	*Formula*	*Precursor*	*Receptor Antagonist*	*Distribution*
acetylcholine (ACh)	$(CH_3)_3N^+—CH_2CH_2—O—\overset{\overset{O}{\|}}{C}—CH_3$	choline	nicotinic d-tubocurare, α-bungarotoxin	neuromuscular junction, autonomic ganglia
			muscarinic atropine, QNB	brain, smooth muscle, glands
norepinephrine (NE)		tyrosine	β propranolol, α phentolamine	midbrain, hypothalamus, cerebellum
dopamine (DA) ...		tyrosine	phenothiazines, butyrophenones pimazide	substantia nigra, basal ganglia, limbic area
serotonin (5HT) ...		tryptophan	cyproheptadine, bromo-LSD	midbrain, raphe
gamma-aminobutyric acid (GABA)	$H_3N^+—CH_2CH_2CH_2—COO^-$	glutamic acid	bicucculine, picrotoxin	throughout brain (inhibitory)
glycine	$H_3N^+—CH_2COO^-$	glycine	strychnine	spinal cord (inhibitory)

QNB = 3-quinuclidinyl-benzilate

transport. Various organelles, enzymes and metabolites have been shown to flow from the cell body to the axon terminal at rates of from 0.1 to 400 mm per day, depending upon the type of tracer substance and the type of axon. Axon terminals have the necessary enzymes to synthesize neurotransmitter molecules from precursors present in the extracellular fluid spaces. Furthermore, several types of nerve terminals are capable of reaccumulating the released neurotransmitter from the synapse through an active reuptake process.

The elements of the synapse are the presynaptic axon termination, with its synaptic vesicles and packets of neurotransmitter and with its reuptake mechanism; the synaptic cleft itself, an intercellular space of 50 to 200 Angstrom units; and the postsynaptic receptor with high-affinity for a specific neurotransmitter. The biosynthetic and metabolic pathways for each transmitter are now well-established, and the enzymes for each step are becoming well characterized. Major advances have been made in the past few years in the identification and characterization of these receptors and in the design of receptor-blocking drugs with high specificity. Table 1 lists the six major transmitter molecules, their primary precursor and the agents used in studies of their receptors. These neurotransmitters, according to several lines of evidence, account for a minority of all the synapses in the brain. Thus, many other small molecules, including amino acids (aspartate, glutamate, taurine), histamine and peptides have been suggested as potential neurotransmitters. In invertebrates, and possibly in man, there are also electronic synapses, fused junctions which do not require chemical transmitters.

As a general rule, there is only one type of chemical neurotransmitter in the synaptic vesicles of any axon terminal. By contrast, neurotransmitters of many types may be released from their respective axons to act upon the dendritic branches of a single receptive neuron. These transmitters may act to excite or to inhibit the receptive cell, and the same neurotransmitter molecule may be excitatory on some cells and inhibitory on other cells.[3] Excitatory transmitters activate ionic conductance (permeability) of the postsynaptic membrane from the resting potassium equilibrium potential to the sodium equilibrium potential.[7,13,14] If depolarization is sufficient to reach the threshold for adjacent electrically excitable portions of the cell membrane, an all-or-none action potential will result. Inhibitory neurotransmitters appear to activate selectively conductance for chloride ion, hyperpolarizing the membrane. Slow postsynaptic potentials of several seconds' duration also have been described, accompanied by increased transmembrane ionic impedance (resistance), rather than conductance changes.

Many cell-cell connections in the brain occur over long distances, from a cell body in one region through its axon to the dendrites of a cell far removed, like the tracts of connections from substantia nigra to the striatum (basal ganglia).

These and many other tracts have been mapped by a remarkable technique pioneered by Falck and Hillarp[15,16] that identifies the axons containing biogenic amine neurotransmitters (norepinephrine, dopamine and serotonin). Each compound gives characteristic fluorescence outlining the tracts containing them, when freeze-dried tissue sections are treated with dry formaldehyde vapor.[17] More recently, immunofluorescent and immunoenzymatic methods have been employed with antibodies directed against biosynthetic enzymes for specific neutrotransmitters.

Specific Neurotransmitter Systems

Specific neurotransmitter substances have been characterized by distribution of the compound itself, activity and distribution of enzymes required for its synthesis and catabolism, presence of specific reuptake into nerve endings from different regions of brain, biochemical and behavioral effects of drugs that inhibit these enzymes or reuptake systems, distribution of receptors for the neurotransmitter, effects of the neurotransmitter when applied by microiontophoresis to the surface of receptor cells and, finally, the congruence of such biochemical observations with clinical-anatomical inferences about brain functions.

Many of the most important experiments were done first or only with systems other than brain. These experimental systems include the neuromuscular junction and the electric organ of the electric eel for acetyl choline; the adrenal medulla and sympathetic ganglia for catecholamines; the squid giant axon and *Aplysia* for ionic changes involved in bioelectric phenomena; and the large neurons of the crayfish for the inhibitory actions of gamma-aminobutyric acid (GABA) and excitatory actions of glutamate.

Acetylcholine (ACh)

This substance was recognized as a neurotransmitter in the 1920's. ACh is the primary transmitter at the neuromuscular junction, in autonomic ganglia, at postganglionic parasympathetic nerve endings, and for motoneuron collaterals to Renshaw cells in the central nervous system (Table 1). It is synthesized in one step from choline and acetyl coenzyme A (CoA) by the enzyme choline acetylase (ChA) in the nerve endings of cholinergic nerves. ACh is inactivated by hydrolysis by acetylcholinesterase (AChE). AChE has been characterized extensively from the electric organ and from brain; potent inhibitors are known, including organophosphorus compounds which are used as insecticides and nerve gases and readily reversible inhibitors, like physostigmine, which are utilized clinically. AChE is distributed widely throughout the brain, associated with synaptosomal, axonal and glial membranes; it is not a useful marker for cholinergic nerves. ACh itself has been extremely difficult to assay, so progress on the identification of cholinergic neurons in the brain has awaited the sensitive assay and immunohistochemical localization of ChA. ChA has highest activity in the caudate nucleus, retina, corneal epithelium and central spinal roots, with very low activity in dorsal spinal roots and cerebellum. No clinically useful inhibitor of ChA is available. The nicotinic receptor for ACh (AChR) has been isolated and characterized in much detail.[18,19] Some of its properties, especially the topography of binding sites for ACh, are very similar to those of AChE; however, experiments with snake venom α-bungarotoxin, which binds irreversibly to cholinergic receptors in neuromuscular junctions and in electric organ preparations, have distinguished AChR from AChE on postsynaptic membranes.

Cholinergic input involving entirely different muscarinic receptors has been demonstrated in the hippocampus, ascending reticular arousal system, caudate nucleus, parts of the thalamus and pyramidal cells of the cerebral cortex.[20] Sensory afferents from auditory, visual, taste, thermal and chemoreceptors also may be cholinergic. The important drugs affecting cholinergic systems in the brain are atropine and scopolamine, which produce a reduction in ACh content in the cerebral hemispheres. However, the amnesic effects are not well explained, since more potent ACh depleting drugs (hemicholinium) have no such behavioral effect. Certain peripherally acting organophosphorus anticholinesterases produce agitation, confusion and slowed intellectual and motor performance in psychological testing.[21] Again the mechanisms are still mysterious.

The most clearly elucidated pathology of cholinergic systems involves patients with myasthenia gravis. The disease appears to be mediated by antibodies directed against the nicotinic ACh receptor in neuromuscular junctions, causing deficiency of receptor sites and the characteristic syndrome of weakness.[22] Administration of anticholinesterase drugs, such as physostigmine, increases the duration of action of released ACh, overcoming the weakness. The similarity between

AChR of electric eels and of the human neuromuscular junction is sufficiently great that purified electric organ AChR has been employed to produce experimental autoimmune myasthenia gravis (EAM) in animals, including monkeys.[23] Lymphocytes from patients with myasthenia gravis have been transformed *in vitro* when cultured with the AChR, and lymphocytes from patients who improved clinically on prednisone gave notably diminished cellular responses.[24] Furthermore, a link to the peculiar relationship of the thymus to myasthenia gravis is provided by evidence that electric organ AChR cross-reacts on both humoral and cellular immune tests with components of calf thymus.[25]

Catecholamines: Norepinephrine (NE) and Dopamine (DA)

Norepinephrine is synthesized from the amino acid tyrosine by hydroxylation to dihydroxyphenylalanine (dopa), decarboxylation to dopamine, and then beta-hydroxylation to NE. Subsequent N-methylation, in adrenal medulla and in olfactory structures, produces epinephrine from NE.[26] NE is ubiquitous in tissues, serving as transmitter for adrenergic vasomotor fibers[11] in all tissues, except placenta and bone marrow, which lack such vasomotor innervation. In the 1940's von Euler identified NE in sympathetic ganglia and peripheral adrenergic nerves,[27] with the highest content of NE in the splenic nerve, almost exclusively containing nonmyelinated adrenergic fibers. NE was identified in mammalian brain, but for several years was thought to reflect only the vasomotor innervation; its concentration is highest in the hypothalamus.

NE-containing neurons in the brain[17] originate from cell bodies in the pons and medulla oblongata of the brain stem; ascending pathways make monosynaptic connections with cortical areas, limbic system (hippocampus, septum and related areas of the "emotional part" of the brain), and hypothalamus; descending pathways make connections with all parts of the spinal cord. The group of neurons most extensively investigated originates in the locus coeruleus and innervates the cerebellar and cerebral cortex and hippocampus. The cells in the locus coeruleus are densely packed and may all be noradrenergic, making electrophysiological recordings and selective stimulation or destruction quite feasible in experimental animals. The terminal networks of this system are very diffuse, with more than 100,000 terminals from an individual monoamine-containing cell body. These diffuse networks are thought to modulate complex phenomena underlying emotional mood, state of alertness, sleep, temperature regulation and neuroendocrine functions.[3,8,28] These central noradrenergic neurons are probably the primary site of action of antianxiety drugs such as benzodiazepines.[3]

Dopamine (DA) is not simply a precursor of NE. Its distribution differs notably from that of NE and it represents more than 50 percent of the total catecholamine content of the brain. The highest concentrations are found in the neostriatum (caudate and putamen of the basal ganglia), nucleus accumbens and olfactory tubercle. Its abundance in the basal ganglia has been related to extrapyramidal motor functions. There are three well-defined dopamine systems:[16,17] (1) nigrostriatal cell bodies are located in the zona compacta of the substantia nigra (the region that degenerates in Parkinson's disease) and project primarily to the neostriatum and the central amygdaloid nucleus; (2) mesolimbic dopaminergic cell bodies are located just dorsal to the interpeduncular nucleus in the ventral tegmental area and innervate the nucleus accumbens and olfactory tubercles (this system may be involved in emotional reactions); (3) tubero-infundibular dopaminergic cell bodies are found in the arcuate nucleus of the hypothalamus and innervate the external layer of the median eminence, stimulating release of luteinizing hormone releasing factor and growth hormone and inhibiting release of prolactin. With the precise localization of DA-containing neurons, it has been feasible to record the activity of these cells with extracellular single-unit techniques;[29] drugs that block the postsynaptic DA receptors (haloperidol, chlorpromazine) cause an increase in firing rate via a negative neuronal feedback loop from cells receiving DA input back to the cell body or dendrites of the DA-containing neurons, as proposed much earlier.[30]

The rate-limiting step in catecholamine biosynthesis is tyrosine hydroxylase (TH), a unique constituent of catecholamine-containing neurons and chromaffin cells. This enzyme disappears completely from renal, salivary gland, vas deferens and cardiac tissue upon chronic denervation of sympathetic nerves. The enzyme is stereospecific for L-tyrosine, requires molecular oxygen, Fe^{++}, and a tetrahydropteridine (folate) cofactor, and is associated with the synaptosome fraction from nerve endings. Inhibitors of TH, such as alpha-

methyl-p-tyrosine, reduce the nerve content of DA and NE and have been used to demonstrate the role of NE in exercise and stress and to treat patients with pheochromocytoma. The second step is mediated by L-aromatic amino acid decarboxylase, which acts on dopa and many other compounds and requires pyridoxal phosphate as cofactor. It is not restricted to neural tissues. Finally, the dopamine-beta-hydroxylase (DBH) is a copper-containing protein requiring molecular oxygen and ascorbate and localized to the membranes of amine storage granules in the nerve endings. Since this enzyme acts on certain related compounds, these substrates can replace NE at noradrenergic nerve endings and function as "false transmitters." One of the compounds that inhibit DBH is disulfiram, upon conversion to the copper chelating agent diethyldithiocarbamate. DBH has been difficult to assay in tissues, because of the presence of endogenous high molecular weight inhibitors. However, the enzyme has been purified from adrenal tissue and used to elicit specific anti-DBH antiserum. Furthermore, the release of NE from nerve endings is accompanied by a stoichiometric release of DBH,[31] so that DBH is found in the plasma and has been studied intensively, though inconclusively, as a marker for sympathetic nervous system activity.[32] The opportunity to assay a key enzyme in the peripheral circulation makes many experiments feasible, but the level of DBH activity seems to be more influenced by genetic factors than by any phenomena related to rate of firing of noradrenergic nerves.

Regulation of this pathway clearly occurs at the TH step, coupling the rate of DA or NE release or postsynaptic action, or both, to the need for additional synthesis.[28] Feedback inhibition by catecholamine products occurs, but other mechanisms of altering the rate of synthesis of the enzyme or its specific activity are probably involved, as well. Circulating hormones, including corticosteroids, thyroid hormones and angiotensin II, can influence NE synthesis.

An important advance in the understanding of catecholamines was the recognition that most of the NE in tissues is located within highly specialized granules in sympathetic and noradrenergic nerve endings and chromaffin cells. The granules contain adenosine triphosphate (ATP) in a molar ratio of NE/ATP = 4/1, presumably bound through salt linkages, DBH, Mg^{++}, Ca^{++}-dependent adenosinetriphosphatase, and a soluble protein chromogranin thought to be involved in the storage process. The amine storage granule protects DA and NE from degradation by monoamine oxidase (MAO), buffers their high ionic concentration, converts DA to NE in noradrenergic endings and serves as a depot for neurotransmitter release upon stimulation. The vesicles are formed in the neuronal cell body and transported to the nerve terminal region; if the axon is ligated or crushed, amine-containing granules can be shown to accumulate progressively on the proximal side of the ligation.[33] The antihypertensive agent reserpine depletes biogenic amines by irreversibly damaging these storage granules.

Metabolism of DA and NE is considerably slower than that for ACh and is mediated by MAO and catechol-O-methyltransferase (COMT). MAO oxidatively deaminates DA and NE to aldehydes, which are then oxidized to the corresponding acids. In the brain, NE is preferentially converted to the corresponding glycol-alcohol; ordinarily, COMT adds a 3-methoxy group to these compounds either before or after MAO oxidation. MAO acts intraneuronally, especially on DA, NE, or serotonin that "leaks" out of the granules; COMT acts within the synaptic cleft. Measurement of DA or NE or their metabolites in urine has provided nothing but controversial results in studies of brain function; at least 90 percent of NE metabolites come from the peripheral sympathetic nervous system and adrenal medulla. Even with measurements in the cerebrospinal fluid, results have been difficult to interpret, presumably due to the variety of dopaminergic and noradrenergic neuronal systems. Quantitatively and clinically, the most important process for terminating the action of DA and NE at synapses is reuptake into the presynaptic nerve ending.[34] Drugs that block reuptake include the tricyclic antidepressants, cocaine and amphetamines. Secondary amine derivatives such as desimipramine have enhanced potency against NE and little inhibition of DA uptake. Specific uptake of 6-hydroxydopamine, a cytotoxic compound, into dopaminergic and, to a lesser extent, noradrenergic neurons has provided a powerful experimental tool for "chemical sympathectomy" in the periphery and in the brain.[35] Studies with 6-hydroxydopamine permit localization by chemical lesions and assessment of behavioral consequences of destruction of DA and NE neurons.

In the past few years, it has been discovered that each of these processes may be investigated

in the peripheral blood. Mitochondrial MAO activity in platelets has at least certain properties in common with monoamine oxidase(s) of brain; COMT activity can be assayed in erythrocytes; and uptake of biogenic amines, particularly serotonin, into platelets mimics reuptake in the brain and is inhibited by similar drugs. In every case, much more detailed analysis is required to prove that the process in these peripheral sites is mediated by enzymes and membrane protein systems determined by the same gene as in brain and functioning in the same manner.

Entirely different classes of compounds block NE and DA receptors. The peripheral NE receptors which mediate cardiopulmonary responses are very well characterized pharmacologically, but not isolated biochemically. Blockers of alpha receptors (phentolamine, phenoxybenzamine) prevent hypertensive responses; blockers of beta receptors (propranolol) prevent increase in heart rate (beta-1) and bronchodilatation (beta-2). New agents which are extremely potent inhibitors of NE binding have been developed recently and tested both *in vivo* and on cultured cell lines *in vitro*. DA receptors are blocked by phenothiazines and butyrophenones, which act as antipsychotic agents, and are stimulated by apomorphine, a potent emetic.

The picture of DA neurons has been complicated by recent evidence strongly pointing to dendritic release of DA onto receptors on the same neuron, thereby inhibiting the firing rate of the neuron.[29,36] Amphetamines and other drugs affect the release and binding.

Serotonin (5-hydroxytryptamine, 5HT)

Since the mid-19th century, serum of clotted blood has been known to contain a substance which caused powerful constriction of smooth muscle organs, hence "serotonin." When the structure was elucidated, the indole ring resembled the structure of the psychedelic drug lysergic acid diethylamide (LSD), which also affected smooth muscle preparations. This relationship led to early theories that serotonin (5HT) in the brain might be involved in mental illness.[37]

5-hydroxytryptamine is synthesized in two steps from tryptophan, in a pathway highly analogous to catecholamine biosynthesis. The rate-limiting step is tryptophan hydroxylase, which requires molecular oxygen, tetrahydrobiopterin and sulfhydryl stabilizing compounds. The activity is sensitive to concentration of substrate, and active transport of tryptophan into the cells is significantly affected by dietary intake and diurnal variation in the plasma concentration.[38] Tryptophan hydroxylase is inhibited by p-chlorophenylalanine, which has been used to treat patients with carcinoid tumors. Subsequent decarboxylation of 5-hydroxytryptophan is mediated by the same L-aromatic amino acid decarboxylase which acts upon L-dopa. Catabolism of 5HT by MAO and reuptake of released 5HT into the presynaptic terminals are analogous to the steps in catecholamine systems. It should be noted that no more than 1 to 2 percent of the total body 5HT is found in the brain; about 90 percent occurs in the enterochromaffin cells of the gastrointestinal tract and 8 to 10 percent in the blood platelets. In rats and mice, but not man, additional serotonin is present in mast cells.

Despite the long history, much remains to be learned about serotonin, including regulation of its biosynthesis, characterization of its reuptake and receptors, description of its behavioral effects and actions of certain drugs. Other indoleamines may be present, as well. The pineal gland, situated outside the blood-brain barrier near the dorsal thalamus, contains high concentrations of 5HT, 50 times that of the whole brain. The metabolic activity of the enzymes related to 5HT synthesis and further conversion to melatonin (5-methoxy-N-acetyl-tryptamine) can be controlled by various external factors, including the neural activity of the sympathetic nervous system (NE) which innervates the pineal gland from the superior cervical ganglion. Cycles of environmental light and darkness determine a diurnal rhythm of both 5HT and melatonin.

Several methods have shown that almost all the serotonin-containing cell bodies in the central nervous system are restricted to a narrow group of neurons, known as the raphe nuclei, in the midportion of the lower pons and upper brain stem. Evidence was derived from classical neuroanatomical studies of the sites of degenerating axons after lesions in the medial forebrain bundle of the hypothalamus; by chemical assays for serotonin after such lesions, and by Falck-Hillarp fluorescence histochemistry after lesions and after loading animals with dietary L-tryptophan. When 5HT is administered by microelectrophoresis onto the surface of potential receptor cells, most respond by decreasing their firing rate.[39] Activation occurs in some limbic areas, but this effect could

be due to inhibition of adjacent inhibitory interneurons.

5HT is involved in temperature regulation, in perception or reaction to sensory stimuli, including pain, and in regulation of the sleep cycle. Electrical stimulation of the raphe nuclei in animals elevates body temperature and interferes with habituation to noise or repetitive sensory stimuli (a la LSD effect). Increase in brain 5HT with MAO inhibitors or intraventricular injection of 5HT or its precursor leads to increased time in slow-wave sleep; diminution in 5HT by ablation of the raphe nuclei or inhibition of 5HT synthesis with p-chloro-phenylalanine has the reverse effect producing insomnia. Several other neurotransmitter systems, especially NE from the locus coeruleus, have been implicated in the regulation of these sleep patterns.[40]

Gamma-Aminobutyric Acid (GABA)

GABA was identified in 1950 as a normal constituent of the mammalian central nervous system. No other tissue, except the retina, had more than a trace of GABA. Generalizing from the demonstrated role of GABA as an inhibitory neutrotransmitter,[41,42] Roberts has hypothesized that neuronal systems controlling behavioral sequences are held in check by inhibitory circuits, many utilizing GABA as neurotransmitter, and that inhibition may be released at appropriate moments either by overriding stimulation or by inhibition of the inhibitory neuron ("disinhibition"). However, much more needs to be learned about the integration of various circuits in specific regions of the brain.

GABA is synthesized from one of several pools of the metabolite glutamic acid in brain by decarboxylation;[43] glutamic acid decarboxylase (GAD) is the rate-limiting step in its formation. Metabolism of GABA involves transamination and oxidation, successively, by GABA-transaminase (GABA-T) and succinic semi-aldehyde dehydrogenase, to succinic acid. The transaminase regenerates alpha-keto-glutarate to provide a "glutamate shunt" without further involvement of the Krebs cycle. The highest concentrations of GABA occur in substantia nigra, globus pallidus, hypothalamus, the inferior and superior colliculi and the dentate nucleus (monkey data),[44] but GABA is present in substantial concentrations throughout the gray matter. Localization of GAD by immunofluorescence has confirmed these findings.[42] Both GAD and GABA-transaminase are dependent on the coenzyme pyridoxal phosphate. Deficiency or pharmacological inactivation of this cofactor can produce epileptiform seizures, associated with a reduction in GABA levels.[45] Rare B6-responsive seizures in childhood may be mediated, at least in part, by this mechanism.

GABA may undergo metabolic transformations to other compounds with functional activity in the brain. Gamma-hydroxybutyrate, formed by reduction of succinic semialdehyde, has anesthetic properties and specifically blocks impulse flow in dopaminergic neurons without any effect on NE, 5HT, or GABA neurons.[46] γ-amino-β-hydroxybutyric acid (GABOB), γ-aminobutyrylcholine, γ-butyrobetaine, γ-guanidinobutyric acid have depressant or excitatory effects on at least some neural systems, and γ-aminobutyrylhistidine (homocarnosine) is one of several dipeptides with significant concentrations in brain but unknown function.

There is no doubt that GABA serves as a potent inhibitory transmitter in the lobster nerve-muscle preparation and in the crayfish stretch receptor.[47] GABA release is proportional to the frequency of stimulation of the inhibitory axons and is blocked by lowered Ca^{++} concentration, which blocks neuromuscular transmission; GABA is present in 100 times higher concentration in inhibitory axons than excitatory axons; the enzymes, a specific GABA uptake mechanism, and GABA receptors, blocked by bicuculline and picrotoxin, all have been demonstrated. In mammalian systems, no such clear-cut evidence for roles of GABA has been gathered.[3,40,45] Compared with the other neurotransmitter systems, GABA lacks specific inhibitors of synthesis, uptake and receptor action and lacks well-defined, readily recorded clusters of GABA-containing neurons. One particularly promising system, however, involves the Purkinje cell axons from the cerebellum, which project onto cerebellar subcortical nuclei adjacent to the fourth ventricle (Deiter's neurons). Cerebellar stimulation increases the amount of GABA released into the ventricular perfusate, and GABA applied iontophoretically to Deiter's neurons induces inhibitory postsynaptic potential changes. Quantitative studies of GABA may be facilitated by the recent development of a radioreceptor assay using synaptosomal membrane fragments and the displacement of ^{3}H-GABA;[48] in fact, this type of assay may be useful for many other neurotransmitters.

Glycine

The simple amino acid glycine is metabolically related to glutamate, serine, folate and glyoxylate pathways and serves as an inhibitory transmitter in the inhibitory interneurons of the spinal cord ventral horn.[49] Iontophoretic application of amino acids in spinal cord preparations suggested that GABA and glycine were inhibitory, hyperpolarizing motoneurons and inducing permeability changes similar to those associated with postsynaptic inhibition. Anoxia of the lumbosacral cord produced by clamping the thoracic aorta destroys the interneurons preferentially, leaving motor neurons intact; analyses in these experiments showed significant decrease of glycine, but not of GABA. Stimulation of dorsal roots causes release of glycine from perfused spinal cord. Finally, strychnine, which blocks postsynaptic inhibition, blocks the action of glycine at presumed receptors.[50] Since chloride and other anions inhibit binding of strychnine to glycine receptors, it is likely that chloride is the ion whose conductance is increased by inhibitory synaptic action of glycine. As in the case of GABA, there are no well-defined neuronal pathways which can be isolated for detailed analysis of the role of glycine.

However, there is highly suggestive evidence that the benzodiazepines, including the two most commonly prescribed drugs in America, diazepam (Valium®) and chlordiazepoxide (Librium®), partly exert their antianxiety, anticonvulsant and muscle-relaxant effects by enhancing polysynaptic inhibitory processes at glycine receptors in the spinal cord, brain stem and thalamus.[51] The rank order of potency for 21 benzodiazepines in displacing ^{3}H-strychnine binding correlated with the rank order of potency in a variety of pharmacological and behavioral tests that predict clinical efficacy. No such correlation could be found for the weaker binding to opiate and cholinergic receptors, though additional sites of action for these drugs were not ruled out. Tetanus toxin also affects spinal cord inhibitory synapses involving glycine, by acting on presynaptic sites; the toxin (tetanospasmin) is transported from peripheral lesions by retrograde intraaxonal transport through motor neurons and across a synapse to the inhibitory nerve terminals.[52]

Other Potential Neurotransmitters

The majority of synapses remains unaccounted for even when all of the techniques presented above are employed to identify synapses utilizing each of the six major neurotransmitters. Certain amino acids occur in uniquely high concentrations in the brain and may serve neurotransmitter roles, as well. Glutamate and aspartate, the dicarboxylic amino acids, have strong excitatory effects in crustacean and mammalian spinal cord tests. Specific antagonists are required to evaluate the role of these compounds at synapses. The biggest problem about these compounds is their ready transformation into related metabolites. Certain complex amino acids, including taurine[54] and cystathionine, occur in unusually high concentrations in mammalian and primate brain; these two might be inhibitory transmitters. Finally, histamine,[55] formed from the amino acid histidine by specific decarboxylation, has some properties suggestive of a neurotransmitter role, including specific uptake into synaptosomes and differential regional concentration, highest in hypothalamus, intermediate in midbrain, and low in cortex and white matter (like the biogenic amines NE and 5HT). A functional role has not yet been delineated.

Neuromodulators: Cyclic AMP, Cyclic GMP, Prostaglandins, Peptides

In addition to the neurotransmitter substances which are released transsynaptically to effect rapid changes in ionic permeability and depolarize or hyperpolarize the postsynaptic membrane, there are other substances whose effects are longer-lasting and seem to modulate the firing rates of postsynaptic cells. It is likely that the two classes of substances are tightly interrelated.

Cyclic AMP

Brain contains extremely high activities of both adenyl cyclase and phosphodiesterase, the enzymes which synthesize and cleave cyclic adenosine monophosphate (AMP). In other tissues, of course, cyclic AMP has proved to be the intracellular mediator of the effects of epinephrine and most polypeptide hormones. In brain, norepinephrine, dopamine and probably serotonin all act by stimulating adenyl cyclase and increasing cyclic AMP concentrations in postsynaptic cells.[56] The adenyl cyclase appears to be intimately associated with the neurotransmitter-specific receptor. Cerebellar Purkinje cells have beta-adrenergic receptors; intracellular recordings reveal that NE hyperpolarizes these membranes, increases membrane resistance, and increases

cyclic AMP.[57,58] Iontophoretically applied cyclic AMP has the same effects, and inhibitors of the phosphodiesterase potentiate the effects of both NE and cyclic AMP. Dopamine-sensitive adenyl cyclases[59] have been demonstrated in rat caudate nucleus and in rabbit sympathetic ganglia. Serotonin increases cyclic AMP levels in rabbit brain slices[60] and, at least in insect neural tissue, stimulates adenyl cyclase activity; blockers of serotonin receptors (bromo-LSD and cyproheptadine) inhibit serotonin stimulation of cyclase activity.[61]

Greengard[56] has proposed a general scheme for amplification of the postsynaptic response by a cascade of reactions mediated by cyclic AMP-dependent phosphorylation of protein kinases; reversible phosphorylation of a membrane protein reversibly alters membrane permeability. The process is reversed by phosphoprotein phosphatases and by phosphodiesterase(s). Such tightly-coupled enzymatic events provide a means to integrate the inhibitory and excitatory influences of multiple synaptic inputs on a given cell. Cyclic AMP may be important also in other intracellular events, including synthesis of RNA and proteins, with long-term trophic effects.

Cyclic GMP

In many systems, there appears to be a balance of action between cyclic guanosine monophosphate (GMP) and cyclic AMP.[56] There is now good evidence in brain that acetyl choline stimulation of muscarinic receptors is mediated intracellularly by increase in cyclic GMP through increased guanyl cyclase activity, opening membrane channels for sodium-potassium exchange. Atropine, the classic muscarinic AChR blocker, prevents the increase in cyclic GMP.

Prostaglandins

The many compounds classified as prostaglandins fall into two series, the E series (extracted into ether) and the F series (extracted by fosphate).[62] In the sympathetic nervous system, prostaglandins of the E series affect both the release of transmitter from adrenergic terminals and the responsiveness of postsynaptic cells to NE. In the mammalian central nervous system, prostaglandins of the F series predominate over E series compounds. The distribution is rather uniform throughout the brain, not suggesting any association with specific pathways. Stimulation of the reticular formation causes release of prostaglandins from the cerebellar cortex, which can be blocked by pretreatment with chlorpromazine. Such stimulant compounds as picrotoxin, pentylene tetrazole and strychnine also release prostaglandins. Prostaglandins may act primarily through interaction with adenyl cyclase.

Substance P

When von Euler and Gaddum were assaying ACh in extracts from brain and intestine in 1931, they detected another pharmacologically active substance which was named substance P (isolated as a powder). This small peptide (ArgProLysProGluGlnPhePheGlyLeuMet-NH_2) is a potent vasodilator and constrictor of smooth muscle, found almost exclusively in the digestive tract and the nervous system.[63] In spinal dorsal root ganglion fibers, which are rich in substance P, this compound has an excitatory action with slow onset and relatively long duration. Substance P is found in high concentration also in the substantia nigra and midbrain structures. The synthetic peptide and antisera against this substance are being tested extensively, as are other peptides isolated from brain. Of these, the most interesting at present are two pentapeptides called enkephalins, which act as agonists at opiate receptors (see below).

Brain-Specific Proteins

In addition to enzymes involved in the biosynthesis and metabolism of neurotransmitter substances and receptors for their action, many other aspects of brain biochemistry have been examined. Most metabolic processes, including DNA, RNA and protein synthesis, much of intermediary metabolism, and the structural proteins in microtubules and microfilaments share the biochemical features of other tissues. It is of special interest, however, that the diversity of RNA messages, determined by hybridization to radioactively-labeled tracer sequences of single-copy DNA, is much greater in brain, especially cerebral cortex, than in other complex organs. This difference is greatest in man[64] and may reflect the complexity of higher cortical functions. The brain also has very active lipid biosynthesis and turnover, especially of phospholipids. Extensive studies of myelin composition and structure have been done, with importance for understanding the degenerative changes in multiple sclerosis and leukodystrophies.[1,2] Glia are especially rich in cortisol-inducible glycerol-3-phosphate dehydro-

genase for lipid synthesis. Figure 1 shows some of the multiple interrelationships among the neurotransmitter compounds and intermediary metabolites and certain substances having unusually high concentration in brain; these include GABA, taurine, cystathionine and N-acetylaspartate.

Two proteins have drawn a large portion of the investigation into special aspects of brain biochemistry. These are the S100 protein,[65] named for its solubility in 100 percent ammonium sulfate, and the 14-3-2 protein,[66] named for its positions on successive elutions from chromatographic columns. Antibodies have been prepared against each protein, assisting in the localization of S100 to glial cells and 14-3-2 to neurons.[67-69] These proteins are not species-specific. A protein termed antigen α is probably the same as 14-3-2.[70]

S100 and 14-3-2 have been useful markers for glial and neuronal cells in methods for bulk separation of these cell types and in characterization of cultured nerve cells *in vitro*. Mouse and human neuroblastoma cell lines and rat glial lines have been employed widely now in analyses of the processes of differentiation of such isolated cells, the appearance and regulation of neurotransmitters and their enzymes and receptors, and the in-

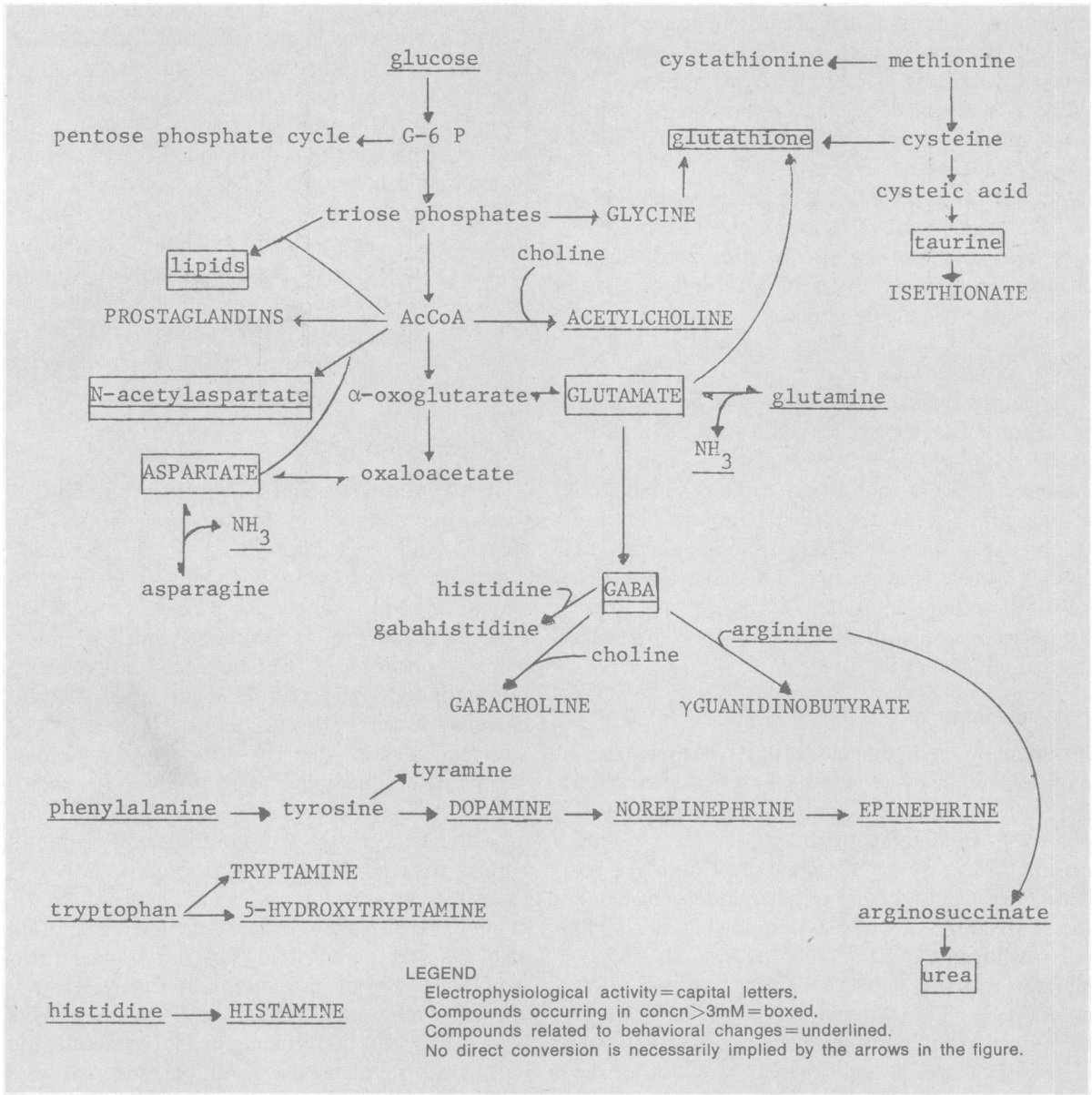


Figure 1.—Various interrelationships among compounds of neuropharmacological interest (adapted from Cooper et al[3] p 54).

teraction of cells of differing types.[71-74] No function is yet known for S100 protein, though S100 does undergo a conformational change when it binds Ca^{++}.[75] Claims have been made that the concentration of S100 is altered in experiments involving memory consolidation,[76] but this result is not widely accepted.[77] No function for the 14-3-2 protein was known either, until Marangos and co-workers showed there to be enolase activity.[78] The physical and chemical properties of this neuron-specific protein coincide with those independently described for the brain-specific isozyme of enolase, an enzyme in the glycolytic pathway. Electrophoretic comparison of brain enolase in 150 human autopsy samples has failed to reveal any differences,[79] just as similar analysis of each of the steps in glycolysis in brain showed that this essential "housekeeping" pathway of energy production is highly conserved.[80] Since there was great hope that the 14-3-2 protein would play some critical role in neuronal function, there may be some disappointment that it turns out to be only a brain-specific isozyme in glycolysis. Other proteins of interest[81] in brain include olfactory bulb protein, glial fibrillary acidic protein, nerve growth factor and the tau factor,[82] which promotes the polymerization of microtubular polypeptide units.

Neurotransmitters and Receptors in Human Psychopathology

The neurochemical studies reviewed thus far have extensive application to affective disorders (depression and manic-depressive illness), schizophrenia(s) and opiate addiction. In these diagnostic categories the probability of heterogeneous underlying mechanisms is so great that one must be extremely cautious in seeking "the cause" or "the treatment" for such disorders.[83] More likely, schizophrenias will prove as heterogeneous as anemias or mental retardation syndromes, with specific therapies. From the investigational point of view, such heterogeneity places a high value on exhaustive study of instructive families with more than one affected person, rather than on a series of unrelated patients.

The Biogenic Amine Hypothesis of Affective Disorders

According to the biogenic amine hypothesis,[84-86] at least some depressions are associated with an absolute or relative deficiency of norepinephrine (NE) or other biogenic amines (serotonin or dopamine) at functionally important receptor sites in the brain; mania, conversely, may be associated with excess of these amines. Pharmacologic agents which deplete NE from nerve terminals (reserpine) or interfere with its biosynthesis (α-methyltyrosine) precipitate depression in susceptible patients treated for hypertension. Drugs that enhance biosynthesis (L-dopa) can induce hypomanic states, and agents that prolong the action of biogenic amines by inhibiting intraneuronal monoamine oxidase (MAO inhibitors) or neuronal reuptake of transmitter released into the synapse (tricyclics, amphetamines) are effective antidepressants. Electroconvulsive shock also acts to increase NE turnover and tyrosine hydroxylase activity.[87] There may be genetically determined individual differences in any of these steps that could be shown by analysis of the effects of the pharmacologic agents.[83] For example, groups of depressed patients have been reported to be differentiated by their response to MAO inhibitors or tricyclics:[88] patients who responded to one class of antidepressant tended not to respond to the other; patients showed the same pattern of responsiveness during a subsequent episode of depression; relatives who had affective disorders shared the pattern of pharmacologic responsiveness or nonresponse. Studies of COMT in erythrocytes[89,90] and MAO in platelets[91,92] have claimed deficiencies or increases of these enzymes in groups of patients with depression. MAO in brain tends to increase with age, possibly contributing to the increasing prevalence of depression with age.[91,93] In fact, despite extensive studies, using various substrates of MAO, the relationships between MAO in platelets and MAO in brain remain to be worked out. Differences in substrate, inhibitor, immunochemical and electrophoretic characteristics have been found, but platelet MAO may still be identical with at least one form of brain MAO's.[94,95] Plasma DBH activity is not altered in patients with affective disorders or by treatment with lithium salts.[96] Lithium, which somehow modulates neurotransmitter effects, is clinically most effective in patients with bipolar manic-depressive illness, but has proved useful in some unipolar patients, as well.

The biogenic amine hypothesis has provided a useful framework for pharmacological observations in affective disorders. However, no primary lesions are known thus far. Direct enzyme assays and metabolite determinations have been fraught with difficulties and inconsistent results.[97] Even

the pharmacological interpretations have been challenged, since drugs that have prompt biochemical actions (tricyclics, for example) require many days for any behavioral change. Therefore, multiple-step compensatory or adaptive biochemical responses to these drugs have been proposed.[98] As an example of endogenous depression, a patient might have a genetic deficiency in COMT, compensation through reduced presynaptic firing rate, adaptive decrease in receptor activity, hyperresponsiveness to tricyclics, and then further adaptive decrease in firing rate and in tyrosine hydroxylase activity. Other studies have tried to distinguish the roles of the different biogenic amines. It is likely, based upon administration of specific precursors (L-dopa and L-tryptophan) and specific inhibitors of biosynthesis (alpha-methyl-tyrosine and p-chlorophenylalanine) that NE (and possibly DA) mediates changes in motor activity and that 5HT mediates changes in mood, as proposed by Lapin.[99] For example, L-dopa can induce hypomanic behavior in bipolar manic-depressives and helps some patients with retarded depressions; however, mood is not improved in patients with unipolar depression.[100,101] L-dopa may even decrease brain 5HT, which analyses of suicides suggest is already low in these patients.[101]

Even with these modifications, many additional influences, especially involving circulating hormones, must not be ignored. The major neuroendocrine disturbances in affective disorders are inhibition of growth hormone responses and hypersecretion of cortisol, both systems under regulation by biogenic amines. Many of these effects have been reviewed recently.[102]

The Dopamine Hypothesis in Schizophrenia

Theories and claims of experimental evidence of biochemical abnormalities in schizophrenia(s) have been legion, including speculations about roles for nearly all of the neurotransmitter compounds or their derivatives.[103,104] Dopamine is the neurotransmitter currently most strongly implicated in the actions of antipsychotic drugs and in the mediation of amphetamine-induced psychosis, the leading experimental model for schizophrenic psychosis. Phenothiazines reverse the primary symptoms of schizophrenia—the thought disorder, disturbances of affect and interpersonal withdrawal—while little affecting delusions, hallucinations, or nonspecific symptoms of anxiety and depression. It must be emphasized, however, that a dopamine hypothesis of antipsychotic drug action does not necessarily imply any primary role of dopamine in the pathogenesis of schizophrenia. There is no direct evidence of dopaminergic hyperactivity in schizophrenic patients; the major metabolite (homovanillic acid) has normal concentration in the cerebrospinal fluid, and serum prolactin, the release of which is strongly influenced by DA neurons, is also normal.[105]

Carlsson and Lindqvist[30] first suggested that phenothiazine and butyrophenone drugs blocked DA receptors, since these drugs increased concentrations of DA metabolites in brain, while the ineffective phenothiazine, promethazine, an antihistaminic drug, produced no such metabolic changes. The potency of antipsychotic phenothiazines is correlated with their conformational similarity to DA as it would fit into DA receptors.

In an attempt to differentiate antipsychotic actions from parkinsonian actions of phenothiazines, thioridazine and clozapine, potent antipsychotic drugs with relatively weak parkinsonian effects, have been investigated extensively in animals. These agents are highly active in several DA test systems.[104] Parallel studies of the affinity of antischizophrenic drugs for muscarinic cholinergic receptors show inverse correlation with their tendency to elicit extrapyramidal side effects;[106] clozapine and thioridazine act on DA receptors just like other phenothiazines but avoid the extrapyramidal side effects by a balancing blockade of ACh receptors in the striatum.

Amphetamines and related stimulant drugs in small doses can exacerbate the symptoms of schizophrenic patients and in large doses can elicit a psychosis clinically indistinguishable from acute paranoid schizophrenia.[107,108] These behavioral changes are reversed by phenothiazines and butyrophenones, the DA receptor-blockers. Amphetamine is now considered a far better mimic of schizophrenia, at least the acute paranoid type, than such psychedelic drugs as LSD, mescaline and dimethyltryptamine. Animal studies show that these stimulant drugs induce stereotyped, repetitive, apparently purposeless activities to the exclusion of normal behaviors. Such activities include sniffing, licking, biting and gnawing—while eating, grooming and sleeping are neglected.[109] Amphetamines, cocaine, phenmetrazine, and L-dopa all elicit a desynchronized electroencephalogram, with associated increase in reticular formation multiple unit activity, a reflection of

central nervous system stimulation. These electroencephalographic effects are entirely different from the hypersynchronous electroencephalographic changes induced by perception-distorting psychedelic agents. Local stimulation of DA receptors and chemical sympathectomy with 6-hydroxy-dopamine provide strong evidence that amphetamine-induced stereotypy is mediated by DA pathways.

This overview of research on schizophrenia presents the current focus on dopamine mechanisms. Work on urinary metabolites, including the "pink spot"[110] (dimethylphenylethylamine), plasma proteins and methylated derivatives of neurotransmitter metabolites in brain all have fallen into the background. However, the transmethylation hypothesis[111] might still apply in certain inborn errors of sulfur metabolism, especially those intrinsic to brain.[112] Given compelling evidence for genetic predisposition to schizophrenia, it is necessary to seek primary gene products, enzymes or polypeptide receptors, rather than just altered metabolic relationships. Dopamine-β-hydroxylase activity in brains of schizophrenics has been reported to be decreased,[113] possibly accounting for deficiency of NE in a NE-mediated reward system.[114] However, these observations have not been confirmable.[104,115] Other investigators have assayed monoamine oxidase (MAO) in platelets, a potential peripheral source of at least one type of brain MAO. MAO activity is reduced in platelets of schizophrenics[116] and in nonschizophrenic identical co-twins of schizophrenic twins.[117] Reduced MAO activity, presumably determined genetically, could cause relative excess of DA (and other biogenic amines), but the effect is not specific for schizophrenia. Assay of brain MAO has failed to show similar deficiency.[118]

Opiate Receptors: Toward an Understanding of Pain and Addiction

Morphine and its congeners have many important medical applications in patients with pain, cough, congestive heart failure, diarrhea and insomnia.[119] The agonist and antagonist compounds are potent and stereospecific, with stringent chemical requirements for activity, suggesting a role for selective receptor sites in the brain. If such receptors exist, there may be some naturally occurring substance in the brain with high affinity for these sites. Remarkable progress has been made in the past five years in demonstrating opiate receptors, peptides with morphine-like activity, and cellular phenomena *in vitro* that mimic addiction.[119,120]

The breakthrough was the preparation of highly radioactive potent opiates, so that tiny concentrations of opiate could be tested for specific, versus nonspecific, binding to extracts of brain or intestine.[121-123] Nonradioactive compounds were tested for displacement of ^{3}H-naloxone or ^{3}H-etorphine, which is 6,000 times more potent than morphine as an analgesic. Then stereospecificity of binding and correlation with analgesic potency were demonstrated; apparent discrepancies were attributable to differences in crossing the blood-brain barrier or metabolic activation (codeine). Sodium ion enhances binding of antagonists and dissociation of agonists in a manner which provides a rapid screen for much-needed mixed agonist-antagonist "nonaddicting" analgesics.[120]

Studies of the regional distribution of binding to opiate receptors in monkey and human brain[123] showed high binding in the amygdala, midbrain, periaqueductal gray, hypothalamus, medial thalamus and caudate nucleus. Within the cerebral cortex, frontal regions bound much more than precentral and postcentral gyri (motor and sensory cortex) or occipital pole; cerebellum and white matter had negligible binding. This distribution corresponds closely with the map of spinothalamic and spinoreticulodiencephalic pain pathways, except for the amygdala.[120] The amygdala is not associated with classical pain pathways, but affective components of pain and euphoric actions of morphine may be mediated by the amygdala and other limbic areas thought to regulate emotional responses. The periaqueductal gray matter is the area in which implantation of morphine most effectively produces analgesia, while the medial thalamus is the site where opiate antagonists best elicit withdrawal symptoms in addicted animals. More detailed mapping of opiate receptors has been achieved by radioautographic localization of binding of ^{3}H-diprenorphine, potent antagonist, in rat brain.[124] Binding was demonstrated in the locus coeruleus, but not in adjacent cells; small doses of morphine are known to slow firing rates of locus coeruleus cells, but not the adjacent cells.[125] In the substantia nigra, radioactivity was concentrated over the zona compacta, but not over the zona reticulata, corresponding to the distribution of dopaminergic cell bodies. Finally, binding was localized in the spinal cord and lower brain stem to the substantia

gelatinosa, an important way station for upward conduction of sensory information relating to pain.

Opiate receptor binding has been demonstrated in the central nervous system of all vertebrates, even primitive fish, but in no invertebrates. The receptor activity is fractionated with nerve endings, stimulating a search for an endogenous neurotransmitter or neuromodulator with specificity for these receptors. The search was promptly successful in several laboratories.[126-128] Brain extracts contain a substance that mimics the ability of morphine to inhibit electrically-induced contractions of smooth muscle preparations and which compete with opiates for specific receptor binding. The regional distribution of this substance, called enkephalin, is similar to that of the opiate receptor. Enkephalin consists of two pentapeptides (TyrGlyGlyPheMet and TyrGlyGlyPheLeu); peptides synthesized to have these sequences have identical biological properties. A third peptide with opiate agonist actions, larger and chemically dissimilar, has been extracted from pituitary. It is of interest that the methionine-enkephalin amino acid sequence occurs also in a protein called β-lipotropin in the pituitary.[126]

A valuable *in vitro* system for analysis of the cellular effects of narcotics has been developed.[74] Hybrid cells derived from fusion of mouse neuroblastoma and rat glial cell lines are rich in opiate receptors (300,000 receptors per cell). Binding of morphine results in a rapid inhibition of adenyl cyclase and a decrease in cyclic AMP in these cells,[129] as well as an increase in cyclic GMP.[130] Continued exposure to morphine caused a compensatory increase in adenyl cyclase activity, termed late positive regulation, restoring cyclic AMP levels to normal. This process makes the cells tolerant to morphine and also dependent upon the narcotic, since withdrawal of the drug or addition of a specific antagonist raises cyclic AMP levels to abnormally high values and secondarily reduces adenyl cyclase activity back to normal levels. The coupled inhibitory and late positive regulatory mechanisms provide a means of activating and deactivating neural circuits hours after the initial event. Methionine-enkephalin mimics these effects of morphine. There are no confounding effects on cell growth, number of receptors or affinity of receptors. Opiates also reverse the stimulation of adenyl cyclase by prostaglandin E_1 and adenine; cells exposed chronically to morphine are tolerant by the criterion that previously effective doses no longer antagonize the prostaglandin stimulation of adenyl cyclase.[129] These hybrid cells can form ACh-containing vesicles, choline acetylase, and apparent synapses and can respond with depolarization to DA. Morphine, in turn, is capable of inhibiting the response to DA.[131] Phosphodiesterase inhibitors (xanthines) produce a pseudoabstinence syndrome and oppose many of the immediate, therapeutic effects of morphine *in vivo*. Studies of cyclic nucleotides in rat brain indicate that opiates rapidly decrease cyclic AMP and increase cyclic GMP *in vivo*.[132]

These receptor assays and *in vitro* cellular systems may permit rapid progress in the elucidation of molecular mechanisms of addiction and of the recognition and affective component of pain.

Parkinson's Disease and Huntington's Disease

These two well-characterized disorders involve the basal ganglia and present with extrapyramidal signs.[133] In Parkinson's disease, discoveries of depigmentation in the substantia nigra and of dopamine deficiency in the striatum[134] set the stage for the demonstration of nigrostriatal dopaminergic fibers arising from cell bodies in the substantia nigra.[16,17] These axons are unmyelinated and the fibers have such small diameters that previous techniques had not uncovered this neuronal connection. The introduction and refinement of L-dopa therapy[135] was one of the most logical developments in medicine. However, many questions remain to be answered:[1,136] the cause of degeneration of cell bodies in the substantia nigra is still unknown; the DA deficiency accounts for akinesia and rigidity, but not for the third cardinal feature, tremor; patients may have notably differing severity of these three signs; and, very importantly, the basis for deterioration of intellectual performance in parkinsonian patients remains to be clarified.

L-dopa administration may introduce many effects beyond simply replacing DA deficits in the cells still able to convert dopa to DA and release DA. One of these effects is choreiform movements, leading to the suggestion that Huntington's disease may be mediated by a genetically-determined excessive responsiveness to normal levels of DA release in the striatum. Use of L-dopa as a provocative test for persons at risk for Huntington's disease is not recommended.[137] In both diseases drugs affecting either cholinergic or dopaminergic systems may be therapeutically effective, so the

balance between these systems and their interaction with GABAergic neurons must be examined and manipulated successfully. Direct assays for enzyme levels in human brain specimens have shown pronounced decrease in tyrosine hydroxylase in the substantia nigra, caudate and putamen, with some decrease in glutamic acid decarboxylase (GAD) and normal choline acetylase (ChA) in Parkinson's disease[138] and pronounced decrease in GAD with lesser loss of ChA in the extrapyramidal structures in Huntington's disease.[138-140] The enzyme loss in Huntington's disease seems to be correlated well with loss of cells and loss of tissue mass, histopathological characteristics of the disease. Huntington's disease is particularly important since it is due to a single abnormal autosomal gene. Patients with this disease have major psychiatric problems and occasionally are diagnosed as paranoid schizophrenics or manic-depressives, contributing to the probable heterogeneity of these major behavioral disorders.

REFERENCES

1. Albers RW, Siegel GJ, Katzman R, et al: Basic Neurochemistry. Boston, Little, Brown & Co, 1972; Siegel GJ, Albers RW, Katzman R, et al, 2nd Ed, 1976 (in press)

2. Lajtha A (Ed): Handbook of Neurochemistry, 7 vols. New York, Plenum Press, 1970

3. Cooper JR, Bloom FE, Roth RH: The Biochemical Basis of Neuropharmacology, 2nd Ed. Oxford Univ Press, 1974

4. Schmitt FO (Ed): The Neurosciences—A Study Program, 3rd Ed. New York, Rockefeller Univ Press, 1974

5. Eccles JC: The Understanding of the Brain. New York, McGraw-Hill, 1973

6. Iversen LL, Iversen SD, Snyder SH (Eds): Handbook of Psychopharmacology, 6 vols. New York, Plenum Press, 1975

7. Hall ZW, Hildebrand JG, Kravitz EA: Chemistry of Synaptic Transmission: Essays and Sources. Newton, Mass, Chiron Press, 1974

8. Myers RD: Handbook of Drug and Chemical Stimulation of the Brain. New York, Van Nostrand Reinhold Co, 1974

9. Gazzaniga MS, Blakemore CS (Eds): Handbook of Psychobiology. New York, Academic Press, 1975

10. Vannucci RC, Plum F: Pathophysiology of perinatal hypoxic-ischemic brain damage. Biology of Brain Dysfunction 3: 1-45, 1975

11. Edvinsson L: Neurogenic mechanisms in the cerebrovascular bed. Autonomic nerves, amine receptors, and their effects on cerebral blood flow. Acta Physiol Scand Suppl 427:1-35, 1975

12. Berl SS, Puszkin S, Wiklas WJ: Actomyosin-like protein in brain. Science 179:441-446, 1973

13. Hodgkin AL, Huxley AF: Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J Physiol 116:449-472, 1952

14. Katz B: Nerve, Muscle and Synapse. New York, McGraw-Hill, 1966

15. Falck B, Hillarp N-A, Thieme G, et al: Fluorescence of catecholamines and related compounds condensed with formaldehyde. J Histochem Cytochem 10:348-354, 1962

16. Dahlström A, Fuxe K: Evidence for the existence of monoamine-containing neurons in the central nervous system. Acta Physiol Scand (Suppl) 62 (232):1-55, 1964

17. Ungerstedt U: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol Scand (Suppl) 367:1-49, 1971

18. Potter LT, Molinoff PB: Isolation of cholinergic receptor proteins. *In* Snyder SH (Ed): Perspectives in Neuropharmacology. New York, Oxford Press, 1972, pp 9-41

19. De Robertis E, Schacht J (Eds): Neurochemistry of Cholinergic Receptors. New York, Raven Press, 1974

20. Yamamura HI, Kuhar MJ, Greenberg D, et al: Muscarinic cholinergic receptor binding: Regional distribution in monkey brain. Brain Res 66:541-546, 1974

21. Karczmar AG: Central cholinergic pathways and their behavioral implications. *In* Clark WG, Del Giudice J (Eds): Principles of Psychopharmacology. New York, Academic Press, 1970, pp 57-86

22. Fambrough DM, Drachman DB, Satyamurti S: Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. Science 182:293-295, 1973

23. Tarrab-Hazdai R, Aharonov A, Silman I, et al: Experimental autoimmune myasthenia induced in monkeys by purified acetylcholine receptor. Nature 256:128-130, 1975

24. Abramsky O, Aharonov A, Teitelbaum D, et al: Myasthenia gravis and acetylcholine receptor. Arch Neurol 32:684-687, 1975

25. Aharonov A, Tarrab-Hazdai R, Abramsky O, et al: Immunological relationship between acetylcholine receptor and thymus: possible significance in myasthenia gravis. Proc Natl Acad Sci U.S. 72:1456-1459, 1975

26. Axelrod J: Noradrenaline: Fate and control of its biosynthesis. Science 173:598-606, 1971

27. von Euler US: Noradrenaline. Springfield, IL, Charles C Thomas, 1956

28. Usdin E, Snyder SH: Frontiers in Catecholamine Research. Proc Third Intl Catecholamine Symposium. New York, Pergamon Press, 1973

29. Bunney BS, Walters JR, Roth RH, et al: Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. J Pharmacol Exp Therap 185:560-571, 1973

30. Carlsson A, Lindqvist M: Effect of chlorpromazine and haloperidol on the formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol (Kbh) 20:140-144, 1963

31. Weinshilboum RM, Thoa NB, Johnson DG, et al: Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. Science 174:1349-1351, 1971

32. Goldstein M, Freedman LS, Ebstein RP, et al: Human serum dopamine-beta-hydroxylase: relationship to sympathetic activity in physiological and pathological states, *In* Usdin E (Ed): Neuropsychopharmacology of Monoamines and their Regulatory Enzymes. New York, Raven Press, 1974, pp 105-119

33. Dahlström A, Häggendal J: Axonal transport of amine storage granules in sympathetic adrenergic neurons. Adv Biochem Psychopharmacol 2:65-93, 1970

34. Iversen LL: Role of transmitter uptake mechanisms in synaptic neurotransmission. Brit J Pharmacol 41:571-591, 1971

35. Thoenen H, Tranzer JP: The pharmacology of 6-hydroxydopamine. Ann Rev Pharmacol 13:169-180, 1973

36. Groves PH, Wilson CJ, Young SJ, et al: Self-inhibition by dopaminergic neurons. Science 190:522-529, 1975

37. Barchas J, Usdin E: Serotonin and Behavior. New York, Academic Press, 1973

38. Wurtman RJ, Fernstrom JD: L-tryptophan, L-tyrosine, and the control of brain monoamine biosynthesis, *In* Snyder SH, (Ed): Perspectives in Neuropharmacology. New York, Oxford University Press, 1972, pp 143-193

39. Bloom FE, Hoffer BJ, Siggins GR, et al: The effects of serotonin on central neurons: Microiontophoretic administration Fed Proc 31:97-106, 1972

40. Jouvet M: The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. Ergeb Physiol 64:166-307, 1972

41. Roberts E, Baxter F, Van Harreveld A, et al: Inhibition in the Nervous System and Gamma-aminobyturic Acid. New York, Pergamon Press, 1960

42. Roberts E, Chase TN, Tower DB (Eds): GABA in Nervous System Function. New York, Raven Press, 1976

43. Baxter CF: Some recent advances in studies of GABA metabolism and compartmentalization, *In* Roberts E, Chase TN, Tower DB (Eds): GABA in Nervous System Function. New York, Raven Press, 1976, pp 61-87

44. Fahn S, Cote LJ: Regional distribution of γ-aminobutyric acid (GABA) in brain of the Rhesus monkey. J Neurochem 15: 209-213, 1968

45. Meldrum BS: Epilepsy and γ-aminobutyric acid-mediated inhibition. Int Rev Neurobiology 17:1-36, 1975

46. Walters JR, Roth RH, Aghajanian GK: Dopaminergic neurons: similar biochemical and histochemical effects of gamma-hydroxy-butyrate and acute lesions of the nigro-neostriatal pathway. J Pharmacol Exp Ther 186:630-639, 1973

47. Iversen LL: The uptake, storage, release, and metabolism of GABA in inhibitory nerves, *In* Snyder SH (Ed): Perspectives in Neuropharmacology. New York, Oxford University Press, 1972, pp 75-111

48. Enna SJ, Snyder SH: A simple, sensitive, and specific radioreceptor assay for endogenous GABA in brain tissue. J Neurochem 26:221-224, 1976

49. Aprison MH, Davidoff RA, Werman R: Glycine: its metabolic and possible transmitter roles in nervous tissue, *In* Lajtha A (Ed): Handbook of Neurochemistry, Vol 3. New York, Plenum Press, 1970, pp 381-397

50. Young AB, Snyder SH: The glycine synaptic receptor: evidence that strychnine binding is associated with the ionic conductance mechanism. Proc Natl Acad Sci USA 71:4002-4005, 1974

51. Young AB, Zukin SR, Snyder SH: Interaction of benzodiazepines with central nervous glycine receptors: possible mechanism of action. Proc Natl Acad Sci USA 71:2246-2250, 1974

52. Price DL, Griffin J, Young Y, et al: Tetanus toxin: direct

evidence for retrograde intraaxonal transport. Science 188:945-947, 1975

53. Johnson JL: Glutamic acid as a synaptic transmitter in the nervous system: A review. Brain Res 37:1-19, 1972

54. Huxtable R, Barbeau A (Eds): Taurine. New York, Raven Press, 1976

55. Snyder SH, Taylor KM: Histamine in the brain: a neurotransmitter? *In* Snyder SH (Ed): Perspectives in Neuropharmacology. New York, Oxford University Press, 1972, pp 43-73

56. Greengard P, McAfee DA, Kebabian JW: On the mechanism of action of cyclic AMP and its role in synaptic transmission. Adv Cyclic Nucleotide Res 1:337-355, 1972

57. Hoffer BJ, Siggins GR, Oliver AP, et al: Cyclic AMP mediates adrenergic synapses to cerebellar Purkinje cells. Adv Cyclic Nucleotide Res 1:411-423, 1972

58. Hoffer BJ, Siggins GR, Oliver AP, et al: Activation of the pathway from locus coeruleus to cerebellar Purkinje neurons: Pharmacological evidence for noradrenergic central inhibition. J Pharm Exp Therap 184:553-569, 1973

59. Kebabian JW, Petzold GL, Greengard P: Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor." Proc Natl Acad Sci USA 69:2145-2149, 1972

60. Kakiuchi S, Rall TW: The influence of chemical agents on the accumulation of adenosine 3′, 5′-phosphate in slices of rabbit cerebellum. Mol Pharmacol 4:367-378, 1968

61. Nathenson JA, Greengard P: Serotonin-sensitive adenylate cyclase in neural tissue and its similarity to the serotonin receptor: A possible site of action of lysergic acid diethylamide. Proc Natl Acad Sci USA 71:797-801, 1974

62. von Euler US, Eliasson R: Prostaglandins. New York, Academic Press, 1967

63. Chang MM, Leeman SE: Isolation of a sialogogic peptide from bovine hypothalamus tissue and its characterization as substance P. J Biol Chem 245:4784-4790, 1970

64. Grouse L, Omenn GS, McCarthy BJ: Studies by DNA-RNA hybridization of the transcriptional diversity of human brain. J Neurochem 20:1063-1073, 1973

65. Hyden H, McEwen B: A glial protein specific for the nervous system. Proc Natl Acad Sci USA 55:354-358, 1966

66. Moore BW: Brain-specific proteins, *In* Schneider DJ, Angeletti RH, Bradshaw, et al (Eds): Proteins of the Nervous System. New York, Raven Press, 1973, pp 1-12

67. Cicero TJ, Cowan WM, Moore BW, et al: The cellular localization of the two brain-specific proteins, S-100 and 14-3-2. Brain Res 18:25-34, 1970

68. Cicero TJ, Ferrendelli JA, Suntzell V, et al: Regional changes in CNS levels of the S-100 and 14-3-2 proteins during development and aging of the mouse. J Neurochem 19:2119-2125, 1972

69. Pickel V, Reis DJ, Marangos PJ, et al: Immunocytochemical localization of nervous system specific protein (NSP-R) in rat brain. Brain Res 106, in press, 1976

70. Bennett GS: Immunologic and electrophoretic identity between nervous system-specific proteins antigen alpha and 14-3-2. Brain Res 68:365-369, 1974

71. Nelson PG: Nerve and muscle cells in culture. Physiol Rev 55:1-61, 1975

72. Matsuzawa H, Nirenberg M: Receptor-mediated shifts in cGMP and cAMP levels in neuroblastoma cells. Proc Natl Acad Sci USA 72:3472-3476, 1975

73. Prasad KN, Gilmer KN: Demonstration of dopamine-sensitive adenylate cyclase in malignant neuroblastoma cells and change in sensitivity of adenylate cyclase to catecholamines in "differentiated" cells. Proc Natl Acad Sci USA 71:2525-2529, 1974

74. Klee WA, Nirenberg M: A neuroblastoma x glioma hybrid cell line with morphine receptors. Proc Natl Acad Sci USA 71:3474-3477, 1974

75. Calissano P, Moore BW, Friesen A: Effect of calcium ion on S-100, a protein of the nervous system. Biochemistry 8:4318-4326, 1969

76. Hyden H, Lange PW: Brain-cell protein synthesis specifically related to learning. Proc Natl Acad Sci USA 65:898-904, 1970

77. Entingh D, Dunn A, Glassman E, et al: Biochemical approaches to the biological basis of memory, *In* Gazzaniga M, Blakemore C (Eds): Hand book of Psychobiology. New York, Academic Press, 1975, pp 201-238

78. Marangos PJ, Zomzely-Neurath C, York C: Determination and characterization of neuron-specific protein (NSP) associated enolase activity. Biochem Biophys Res Commun 68:1309-1316, 1976

79. Omenn GS, Chen SH: Electrophoretic screening for variation in brain-specific enolase (neuron-specific protein). (In preparation)

80. Cohen PTW, Omenn GS, Motulsky AG, et al: Restricted variation in the glycolytic enzymes of human brain and erythrocytes. Nature New Biology 241:229-233, 1973

81. Schneider DJ, Angeletti RH, Bradshaw RA, et al (Eds): Proteins of the Nervous System. New York, Raven Press, 1973

82. Weingarten MD, Lockwood AH, Hwo S, et al: A protein factor essential for microtubule assembly. Proc Natl Acad Sci USA 72:1858-1862, 1975

83. Motulsky AG, Omenn GS: Biochemical genetics and psychiatry, *In* Fieve RR, Rosenthal D, Brill H (Eds): Genetic Research in Psychiatry. Baltimore, Johns Hopkins University Press, 1975, pp 3-14

84. Bunney WE Jr, Davis JM: Norepinephrine in depressive reactions: A review. Arch Gen Psychiat 13:483-494, 1965

85. Schildkraut JJ, Kety SS: Biogenic amines and emotions. Science 156:21-30, 1967

86. Schildkraut JJ: Neuropsychopharmacology and the affective disorders. N Engl J Med 281:197-201; 248-255; 302-308, 1969

87. Mussachio JM, Julou L, Kety SS, et al: Increase in rat brain tyrosine hydroxylase activity produced by electroconvulsive shock. Proc Natl Acad Sci USA 63:1117-1119, 1969

88. Pare CMB, Mack JW: Differentiation of two genetically specific types of depression by the response to antidepressant drug. J Med Genet 8:306-309, 1971

89. Cohn CK, Dunner DL, Axelrod J: Reduced catechol-O-methyl transferase activity in red blood cells of women with primary affective disorder. Science 170:1323-1324, 1970

90. Dunner DL, Cohn CK, Gershon ES, et al: Differential catechol-O-methyl-transferase activity in unipolar and biopolar affective illness. Arch Gen Psychiat 25:348-353, 1971

91. Nies A, Robinson DS, Ravaris CL, et al: Amines and monoamine oxidase in relation to aging and depression in man. Psychosomatic Med 33:470, 1971

92. Murphy DL, Weiss R: Reduced monoamine oxidase activity in blood platelets from bipolar depressed patients. Am J Psychiat 128:1351-1357, 1972

93. Omenn GS, Cheung S: Pharmacogenetic analysis of monoamine oxidase in human and macaque brain. Abstr 7th Ann Mtg Amer Soc Neurochem, 1976

94. Costa E, Sandler M (Eds): Monoamine Oxidases—New Vistas. New York, Raven Press, 1972

95. Collins GGS, Sandler M: Human blood platelet MAO. Biochem Pharmacol 20:289-296, 1971

96. Levitt M, Mendlewicz J: A genetic study of plasma dopamine β-hydroxylase in affective disorder. Mod Probl Pharmacopsychiatry 10:89-98, 1975

97. Usdin E (Ed): Neuropsychopharmacology of Monoamines and their Regulatory Enzymes. New York, Raven Press, 1974

98. Segal DS, Kuczenski R, Mandell AJ: Theoretical implications of drug-induced adaptive regulation for a biogenic amine hypothesis of affective disorder. Biol Psychiat 9:147-159, 1974

99. Lapin IP, Oxenkrug GF: Intensification of the central serotonergic processes as a possible determinant of the thymoleptic effect. Lancet 1:132-136, 1969

100. Goodwin FK, Murphy DL, Brodie HKH, et al: Levadopa: Alterations in behavior. Clin Pharmacol Ther 12:383-396, 1971

101. Sachar EJ, Coppen AJ: Biological aspects of affective psychoses. Biology of Brain Dysfunction 3:215-245, 1975

102. Sachar EJ: Hormones, Behavior and Psychopathology. New York, Raven Press, 1976

103. Kety SS, Matthysse S (Eds): Prospects for Research in Schizophrenia. Neurosciences Res Prog Bull 10:370-507, 1972

104. Matthysse S, Sugerman J: Neurotransmitter theories of schizophrenia, *In* Iversen LL, Iversen SD, Snyder SH (Eds): Handbook of Psychopharmacology. New York, Raven Press (In press)

105. Meltzer HY, Sachar EJ, Frantz AG: Serum prolactin levels in unmedicated schizophrenic patients. Arch Gen Psychiat 31:564-569, 1974

106. Snyder SH, Greenberg D, Yamamura HI: Antischizophrenic drugs; affinity for muscarinic cholinergic receptor sites in the brain predicts extrapyramidal effects. J Psychiat Res 11:91-95, 1974

107. Angrist BM, Gershon S: The phenomenology of experimentally induced amphetamine psychosis—Preliminary observations. Biol Psychiat 2:95-107, 1970

108. Snyder SH, Banerjee SP, Yamamura HI, et al: Drugs, neurotransmitters, and schizophrenia. Science 184:1243-1253, 1974

109. Randrup A, Munkvad I: Pharmacology and physiology of stereotyped behavior. J Psychiat Res 11:1-10, 1974

110. Friedhoff AJ, Van Winkle E: The characteristics of an amine found in the urine of schizophrenic patients. J Nerv Ment Dis 135:550-555, 1962

111. Omenn GS: Inborn errors of metabolism: Clues to understanding human behavioral disorders. Behavior Genet 6:263-284, 1976

112. Osmond H, Smythies J: Schizophrenia: A new approach. J Ment Sci 98:309-315, 1952

113. Wise CD, Baden MM, Stein L: Post-mortem measurement of enzymes in human brain: evidence of a central noradrenergic deficit in schizophrenia. J Psychiat Res 11:185-198, 1974

114. Stein L, Wise CD: Possible etiology of schizophrenia: Progressive damage to the noradrenergic reward system by 6-hydroxydopamine. Science 171:1032-1036, 1971

115. Wyatt RJ, Schwartz MA, Erdelyi E, et al: Dopamine β-hydroxylase activity in brains of chronic schizophrenic patients. Science 187:368-370, 1975

116. Murphy DL, Wyatt RJ: Reduced MAO activity in blood platelets from schizophrenic patients. Nature 238:225-226, 1972

117. Wyatt RJ, Murphy DL, Belmaker R, et al: Reduced

monoamine oxidase in platelets—A possible genetic marker for vulnerability to schizophrenia. Science 179:916-918, 1973

118. Wyatt RJ, Belmaker R, Murphy L: Low platelet monoamine oxidase and vulnerability to schizophrenia. Mod Probl Pharmacopsychiat 10:38-56, 1975

119. Snyder SH, Pert CB, Pasternak GW: The opiate receptor. Ann Intern Med 81:534-540, 1974

120. Snyder SH: Opiate receptor in normal and drug altered brain function. Nature 257:185-189, 1975

121. Terenius L: Characteristics of the receptor for narcotic analgesics in synaptic membrane fractions from rat brain. Acta Pharmacol Toxicol 33:377-384, 1973

122. Simon EJ, Hiller JM, Edelman I: Stereospecific binding of the potent narcotic analgesic ^{3}H-etorphine to rat brain homogenate. Proc Natl Acad Sci USA 70:1947-1949, 1973

123. Kuhar MJ, Pert CB, Snyder SH: Regional distribution of opiate receptor binding in monkey and human brain. Nature 245: 447-450, 1973

124. Pert CB, Kuhar MJ, Snyder SH: Autoradiographic localization of the opiate receptor in rat brain. Life Sci 16:1849-1854, 1975

125. Korf J, Bunney BS, Aghajanian GK: Noradrenergic neurons: morphine inhibition of spontaneous activity. Eur J Pharmacol 25:165-169, 1974

126. Hughes J, Smth TW, Kosterlitz HW, et al: Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258:577-579, 1975

127. Terenius L, Wahlström A: Search for an endogenous ligand for the opiate receptor. Acta Physiol Scand 94:74-81, 1975

128. Pasternak GW, Goodman R, Snyder SH: An endogenous morphine-like factor in mammalian brain. Life Sci 16:1765-1769, 1975

129. Sharma SK, Klee WA, Nirenberg M: Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. Proc Natl Acad Sci USA 72:3092-3096, 1975

130. Traber J, Gullis R, Hamprecht B: Influence of opiates on the levels of adenosine 3',5'-cyclic monophosphate in neuroblastoma x glioma hybrid cells. Life Sci 16:1863-1868, 1975

131. Myers PR, Livengood DR, Shain W: Effect of morphine on a depolarising dopamine response. Nature 257:238-240, 1975

132. Collier HOJ, Roy AC: Hypothesis: Inhibition of E-prostaglandin-sensitive adenyl cyclase as the mechanism of morphine analgesia. Prostaglandins 7:361-376, 1974

133. Pincus JH, Tucker G: Behavioral Neurology. New York, Oxford Univ Press, 1974

134. Hornykiewicz O: Dopamine (3-hydroxytyramine) and brain function. Pharmacol Rev 18:925-964, 1966

135. Markham CH, Treciokas LJ, Diamond SG: Parkinson's disease and levodopa—A five year followup and review. West J Med 121:188-206, 1974

136. Barbeau A: Biology of the striatum. Biology of Brain Dysfunction 2:333-350, 1974

137. Omenn GS, Motulsky AG: Pharmacogenetics and mental disease. Psychol Med 4:125-129, 1974

138. McGeer PL, McGeer EG: Enzymes associated with the metabolism of catecholamines, acetylcholine and GABA in human controls and patients with Parkinson's disease and Huntington's chorea. J Neurochem 26:65-76, 1975

139. Bird ED, Iversen LL: Huntington's chorea—Post-mortem measurement of glutamic acid decarboxylase choline acetyltransferase and dopamine in basal ganglia. Brain 97:457-472, 1974

140. Stahl WL, Swanson PD: Biochemical abnormalities in Huntington's chorea brains. Neurology 24:813-819, 1974

Candid Comments on Managing Moniliasis

The most frequent thing we see in the office is some form of moniliasis or low grade fungus infection. And in such situations, you've got to review many things. You've got to review such things as whether they [female patients] wear panty hose, whether they use colored toilet tissue, whether their bowel hygiene is correct, whether they like to sit in the tub for long periods of time or whether they like to sit around in a wet bathing suit for long periods of time. . . . The girls who get this should have their panties washed thoroughly and then boiled . . . that's the best way to try to correct it. Others caution, of course, about the use of vaginal sprays—the labial sprays. All of these things have been a boon to our business . . .

—J. BROOKS HOFFMAN, MD, *Greenwich, CT*
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